Asian Journal of Agriculture and Allied Sciences

Volume 7, Issue 1, Page 56-71, 2024; Article no.AJAAS.12617

Evaluation of Chlorophyll and Carotenoid Contents in Tropical Wheat Varieties under Different Irrigation Regimes

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Authors' contributions

This study was carried out with collaboration between all authors. Author MSW conducted the field and laboratory work, conducted the analysis and draft the manuscript. Author SDA coined the idea and designed the experiment. Author BA conducted the laboratory protocols. Author AMG was part of the idea and similarly supervised the work. Author SPB was also part of the supervisory committee and he also edited the final draft. All authors read and approved the final manuscript.

Article Information

DOI:<https://doi.org/10.56557/ajaas/2024/v7i142>

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://prh.ikprress.org/review-history/12617>

> *Received: 17/10/2024 Accepted: 20/12/2024 Published: 27/12/2024*

Original Research Article

ABSTRACT

Wheat is one of the most important staple food crops in the world. The demand for wheat is expected to rise by 60% in developing countries by the year 2050 due to the increased human population. For the farmers to feed the growing population, the production of wheat must be

Cite as: Waziri, M.S., S.D. Abdul, B. Apagu, A.M. Gani, and S.P. Bako. 2024. "Evaluation of Chlorophyll and Carotenoid Contents in Tropical Wheat Varieties under Different Irrigation Regimes". Asian Journal of Agriculture and Allied Sciences 7 (1):56-71. https://doi.org/10.56557/ajaas/2024/v7i142.

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effectively increased by at least 70%. Despite this threat, the average yield in most wheat-producing regions is seriously reduced by abiotic factors such as drought. Boosting wheat production depends on a higher yield, rather than an increased cropping area. Improving the existing wheat varieties and developing new varieties that will be resistant to water stress is key. This study was conducted to evaluate the changes in chlorophyll and carotenoid contents under two irrigation regimes, to see the effects of their changes on the yield of tropical wheat varieties. The experiment was laid using a split plot with three replications. Two irrigation regimes were the main plot while ten wheat varieties were the subplots. Changes in chlorophyll A and B as well as carotenoids were evaluated. Combined results analysis shows significant changes in all the three pigments when irrigation interval was increased from 5-15 days. While chlorophyll A and B contents increased under 5 days irrigation, carotenoid increased under 15 days irrigation. Chlorophyll A and B were found to be positively correlated with yield in all the tropical wheat varieties. The varieties KAUZ-9, ATTILA-7, and TEVEE'S were found to have better results and were recommended for planting in water deficit environments.

Keywords: Drought resistance; photosynthetic pigment; water stress; yield.

1. INTRODUCTION

Wheat (*Triticum aestivum* L.,) belongs to the family Poaceae (Gramineae), subfamily pooideae. It is commonly called bread wheat and is a cold-loving crop, that is widely spread around the world, growing in arctic and humid regions as well as the tropical highlands. Wheat is one of the most important staple food crops in the world, considered to be one of the main sources of human nutrition [1]. The demand for wheat is expected to rise by 60% in developing countries by the year 2050 [2], because of the increased population. For the farmers to feed the growing population, the production of wheat must be effectively increased by at least 70% [3,4,5]. Despite this threat, the average yield in most wheat-producing regions is seriously reduced by abiotic factors such as drought [6,7].

In Nigeria for example, wheat-producing regions are located in the northern part of the country. States like Bauchi, Borno, Jigawa, Kano, Kebbi, and Zamfara produced 70-80% of the wheat grains. Most of these states are characterized as Arid regions, where rainfall is erratic and unpredictable. A report by Ashraf [8], shows that 30-60% of the water applied to crops in arid areas is lost through evaporation, this made even production of crops using irrigation farming more difficult in the areas. The country has been struggling for a long to meet the local wheat demand of its population. With a population of about 200 million people, the country is the second-largest wheat consumer in sub-Saharan Africa after South Africa [9]. Nigeria's annual wheat production stood at 300,000 metric tons [10], while the demand was estimated to be 4million metric tons. There is a

gap of 3.7 million metric tons which is compensated through importation. Given this context, there is an urgent need to increase wheat production in Nigeria by 97% to meet the local demand. Boosting wheat production in Nigeria will depend on higher yield as seen in some countries, rather than an increase in cropping area. The best way is by improving the existing wheat varieties and developing new varieties that will be resistant to abiotic stress especially water stress [11].

In recent years many researchers have attempted to understand the physiological factors connected with drought in wheat [12]. Drought affects almost all the operational systems in wheat resulting in low photosynthetic activities and hindering growth and yield [13]. Enhancing the physiological traits in wheat could improve adaptation to drought stress and increase yield in the crop [14]. Studies have confirmed the contribution of chlorophyll in wheat adaptation to drought conditions [15]. High chlorophyll and carotenoid contents have been reported to have a significant physiological role in crop performance under water stress conditions [16,17]. The chlorophyll content is an indicator of the photosynthetic capability of plants [18]. Plants maintain survival with the aid of the energy from sunlight to produce food (carbohydrate) by combining carbon dioxide and water.

At the moment the actual changes that enable water-stress resistance remain a poorly understood area in wheat physiology and breeding [19]. Wheat scientists are finding it difficult to create new varieties that will perform in water deficit areas [20]. Although there is various work on changes of chlorophyll content under water stress condition in wheat crop [15,16,17], there are no any such work done on these 10 varieties in the tropical region of Nigeria. Therefore, this study aims at evaluating the changes in chlorophyll and carotenoid contents of (10) tropical wheat varieties under two (2) irrigation watering regimes and their effect on yield and yield-related traits.

2. MATERIALS AND METHODS

2.1 Study Site

The study was conducted at the Abubakar Tafawa Balewa University Bauchi. Bauchi State is situated in the northeast zone of Nigeria. Geographically the state is located between latitudes 9°30' and 12°30' North of the equator, and between longitudes 8°45' and 11°0' East of the Greenwich meridian, it spans between two unique vegetation zones, namely, the Sudan savannah and the Sahel savannah (GPS Coordinate).

2.2 Source of Seeds

A set of ten (10) tropical wheat variety seeds were used, these seeds were originated, tested, and released in Nigeria by Lake Chad research institute (LCRI) in Borno state Nigeria. The pedigree of the seeds is shown in Table 1, these seeds have been widely accepted by wheat farmers in the region because they are available and affordable to the farmers.

Before planting, the soil sample was collected at
0-15cm depth from the site for the depth from the site for the physiochemical analysis presented in Table 2.

Table 2. Physiochemical properties of the experimental site soil at Abubakar Tafawa Balewa University Bauchi, Bauchi State, Nigeria

Key: E.C- electrical conductivity, CaCO³ -calcium carbonate, Mg2+ -magnesium, K⁺ -potassium N-nitrogen, CECcation exchange capacity, O.C-organic carbon, OM-organic matter, P- phosphorus, WHC-water holding capacity

2.3 Experimental Design

The experiment was laid using a split-plot design. Irrigation scheduling was the main plot, while variety is the subplot. The land was cleared, plowed, harrowed, leveled and plots measuring 1.5 x 3m were constructed with wide water channels in between each main plot. Each net plot consists of 4 rows of 3m long with 25cm space between row and 15 cm spacing within the plot. 1m and 1.5m spacing was maintained between treatments and replicates respectively. Planting was done on the 15th of November for two seasons, that is the 2018/2019 and 2019/2020 dry farming season. The seeds sowing was at the rate of 120 kg/ha using hand drilling at 2-3 cm depth as done by Sokoto and Abubakar [21]. After sowing, all plots were irrigated with 15 liters of water immediately for the proper establishment of the seedlings as done by Lado, [22]. Check basin irrigation method was used; subsequently watering was done following the irrigation scheduling at 5 days intervals (Treatment 1), and 15 days intervals (Treatment 2). Weeds were removed manually with the hand at certain intervals. All agronomic practices used in the wheat-growing field were strictly followed in all the plots.

2.4 Data Collection

2.4.1 Estimation of chlorophyll and carotenoid

The content of chlorophyll A, chlorophyll B, and carotenoid were estimated at vegetative and reproductive stages of growth following the methods described by Rahimi et al. [23]. Leaf samples were collected from 9:30 to 10:00 am from the third fully expanded leaf from the top. Half gram of fresh sample material was poured in a porcelain mortar, then split using 95% ethanol and crushed. 20ml of 80% acetone was added to the sample and centrifuged at 6,000 rpm for 10 min. The upper isolated extract from the centrifugation was transferred into a glass balloon. The number of samples inside the balloon was poured into a cuvette. Absorbance rate was read in a spectrophotometer at wavelengths of 663nm for chlorophyll A and 645nm for chlorophyll B and 470 nm for carotenoids. Readings were taken three times to avoid bias. Using the following formula of Arnon, [24] chlorophyll A, B, and carotenoids were calculated in milligrams per gram of sample fresh weight.

Chlorophyll a = (19.3×A663-0.86×A645) V/100W Chlorophyll b = (19.3×A645-3.6×A663) V/100W

Carotenoids = 100 (A470)-3.27 (mg chl. a)-104 (mg chl. b)/227

Where: V = Volume of filtrate. A = Absorption of light at wavelengths of 663, 645 and 470 nm, for chlorophyll a, b, and carotenoids respectively. W = Sample fresh weight in grams.

Determination of yield and yield-related traits: Ten (10) plants were randomly selected from the two middle rows and tagged for the recording of the following traits:

- 1. **The number of spikes:** This was determined by counting all spikes from the tagged plants from each plot.
- 2. **Spike Length (cm):** this was measured using a meter ruler from the base to the tip of the spike excluding awns at maturity.
- 3. **The number of grains per spike:** was counted from the spikes of the tagged sample plants that were used to determine the previous parameters.
- 4. **Weight of 1000 grains (g):** was determined by weighing 1000 seed grains from the tagged sample plants at each plot and recording it in kg.
- 5. **Grain Yield (t/ha):** was achieved by weighing the seed from each plot and expressed into hectare in kilogram before converting it into tons per hectare (t/h)

2.5 Statistical Analysis

All the data for the two growing seasons were analyzed using STATISTICS ANALYTICAL SOFTWARE VERSION 8.0. Analysis of variance (ANOVA) for split-plot design was used to calculate means, standard errors, and significant difference between treatments. Probability of significance was used to see the significance and interaction between treatments at $P \le 0.05$ and P < 0.01 levels of significance. Standard error (SE) was used to compare means. Pearson's correlation coefficient of photosynthetic pigments and yield-related traits and yield were also analyzed.

3. RESULTS

Fig. 1 shows the changes in chlorophyll A content in the tropical wheat varieties at vegetative and reproductive stages of growth when subjected to two different irrigation watering regimes. From the figure, the content of chlorophyll A increases with an increase in irrigation, 5 days irrigation regime has the highest

Fig. 1. Changes in chlorophyll A content at vegetative and reproductive stages of growth in tropical wheat varieties under different irrigation regimes

Fig. 2. Chlorophyll A content at vegetative and reproductive stages of growth in tropical wheat varieties

Fig. 3. Changes in chlorophyll B content at vegetative and reproductive stages of growth in tropical wheat varieties under different irrigation regimes

Fig. 4. Chlorophyll B content at vegetative and reproductive stages of growth in tropical wheat varieties

Fig. 5. Changes in carotenoid content at vegetative and reproductive stages of growth in tropical wheat varieties under different irrigation regimes

Fig. 6. Carotenoids content at vegetative and reproductive stages of growth in tropical wheat varieties

contents at both the two growth stages compared to 15 days. Similarly, chlorophyll A is higher during the vegetative growth stage compared to the reproductive stage. This shows that the chlorophyll A content decreased as the varieties reaches the reproductive stage under both irrigation regimes.

Fig. 2 show the changes in chlorophyll A content in the tropical varieties, it follows the same trend as that of the Fig. 1. The contents of chlorophyll A were much higher during the vegetative stage than during the reproductive growth stage in all the tropical wheat varieties. Among all the varieties KAUZ-9 has the highest content, followed by ATTILA-7 and TEVEE'S. The least content of chlorophyll A was recorded in NORMAN at all the growth stages.

Fig. 3 show how the content of chlorophyll B changes with changes in irrigation regimes, the figure shows an increased amount of chlorophyll B when irrigation is applied following 5 days irrigation regime at both growth stages, there is also an increased level of chlorophyll B during vegetative stage compared to reproductive stage.

In Fig. 4 the varieties show a similar response with that of Fig. 2, the chlorophyll B content was found to be higher at vegetative compared to the reproductive stage of growth. Likewise, KAUZ-9, TEVEE'S, and ATILLA-7 gave the highest result compared to the other varieties.

Fig. 5 show the changes in carotenoid contents under the two irrigation regimes, the figure gave an opposite trend with that of the Figs. 1 and 3. Here the levels of carotenoids in both reproductive and vegetative growth stages were higher when irrigation was applied following 15 days irrigation interval as opposed to 5 days in chlorophyll A and B. This shows carotenoids increase with an increase in water stress. Also, the carotenoid level was higher during the reductive stage compared to the vegetative growth stage. This shows that as chlorophyll is increasing, carotenoids is decreasing.

The level of carotenoid contents in all the varieties is also higher at the reproductive stage than the vegetative stage (Fig. 6). The varieties KAUZ-9, ATILLA-7, TEVEE'S, and REBWA-19 gave the highest content, while USHER and GOUMRIA were the least in all the two growth stages.

Table 3 shows the effects of varying irrigation regimes and varieties as well as their interactions

on some yield components and yield of tropical wheat varieties grown during the 2018/2019 dry farming seasons. Results show a highly significant (p<0.01) effect on irrigation regimes of all the measured yield components. 5 days irrigation regime was consistently higher compared to 15 days. Therefore, the values of these yield components increase as irrigation increased. Results also show a significant difference (P<0.05) between the varieties, their interactions were also highly significant $(p<0.01)$ except that of spike length which shows no significance. The response of the varieties to the different irrigation regimes varies significantly (p<0.05), number of spikes per stand was almost significantly similar in all the varieties, however, REBWA-19 has the highest mean value of 14.44 while the least (13.06) comes from IMAM. KAUZ-9 and TEVEE'S have the longest spike length (12.01 and 11.68 respectively), followed by ATTILA-7 (10.64), REBWA-19 (10.64), and USHER (10.68). The shortest spike length (7.86) comes from SERI-82. The highest values for the number of grains (57.39, 55.67, and 55.44) were obtained from KAUZ-9, USHER, and TEVEE'S respectively, while the least values (43.11, 42.29, and 41.56) come from NORMAN, GOUMRIA, and SERI-82 respectively. The value of thousand-grain weight from TEVEE'S (45.86) was significantly (P<0.05) higher than the values from the rest of the varieties, the least values (37.77 and 37.67) of thousand-grain weight was recorded from NORMAN and SERI-82 respectively. KAUZ-9 and TEVEE'S have the highest values (4.34 and 4.18) of grain yield followed by ATTILA-7 (3.69). The least grain yield (2.74) was recorded from SERI-82.

Table 4 show the correlation coefficients among the photosynthetic pigments, yield, and yieldrelated traits of tropical wheat varieties. The table shows a positive correlation between chlorophyll A and B with yield and all the yieldrelated traits observed in this study except for the number of spikes that shows a negative correlation with chlorophyll B. On the contrary, the table shows a negative correlation between carotenoid and yield and all the yield-related traits measured during the study. Therefore, from the table, it shows that increase in the amount of chlorophyll A and B led to a significant increase in yield-related traits which also increased grain yield in all the tropical wheat varieties, while increased content of carotenoids negatively affects the yield-related traits and has shown to reduce the grain yield of the tropical wheat varieties.

Table 3. Influence of irrigation regimes on yield related traits and yield of tropical wheat varieties grown during 2018/2019 and 2019/2020 dry farming seasons

*Means within each column followed by the same letter(s) are not significantly different at (P≤0.05) using SE. NS=Not Significant; **=significant at 1%*

*ns= not significant, *= significant at 5% probability level, **= significant at 1% probability level, CHL A = Chlorophyll a, CHL B= Chlorophyll b, CRT=, GYD= Grain yield, NGS= Number of grains, NSPK= Number of per spikes, SPL= Spike length*

4. DISCUSSION

In Nigeria, wheat production is done mostly in the Northern part of the country, where rainfall is erratic and unpredictable. This constitutes a major challenge in meeting the local demand for wheat by the farmers. Wheat farmers opted for an irrigation farming system in the country. Adopting certain approaches in wheat production using irrigation will help to increase wheat production and help in conserving the overexploited soil moisture [25,26]. One of the approaches that help is enhancing the photosynthetic ability of crops growing under

water stress conditions, is increased chlorophyll contents [27,28,29]. Results from this study indicated a reduced level of both chlorophyll A and B contents under water stress conditions. This shows that water stress affects the photosynthetic capacity of the wheat crop since chlorophyll content is a good indicator of the photosynthetic capability of plant tissues [16,18,30,31]. Chlorophyll A and B contents were also found to be higher during the vegetative stage of growth compared to the reproductive stage. This may be attributed to the increased level of stress during the reproductive stage because the ill effects of drought worsen with duration, intensity, and growth stage [11,15,32,33]. It has also been reported that water stress in wheat is more severe during the reproduction stage and reduced the photosynthetic ability of the crop [19,34,35,36]. This is because of the severe competition on nutrients during the reproductive stage compared to the vegetative stage of growth [37,38,39], also the chlorophyll might have been utilized in grain production during the reproductive stage [20,39].

The number of spikes, spike lengths, grain per spike, and thousand-grain weight were all found to be reducing when irrigation was delayed to 15 days interval compared to 5 days interval. The same observation was reported by Abdul Latief et al. [36], who observed a rapid increase of up to 50% of these yield-related traits with an increase in soil water content. Reduction in the yield-related traits observed under 15 days irrigation regime in this study may be due to a significant decrease in chlorophyll contents under 15 days irrigation regime. Similar findings were also reported by Semcheddine, and Hafsi [40].

A positive correlation was observed between chlorophyll A and B contents with yield-related components of the varieties in this study, this may be the reason for increased grain yield in the varieties with a higher level of chlorophyll. Similar findings were also reported by Bijanzadeh et al., [41]. Sexena et al. [42], and Rahimi et al. [23]. Othmani et al. [31] said grain yield in wheat depends on the transfer of assimilates and the remobilization of the assimilates in the vegetative organs. Dry matter accumulation post-anthesis determined the yield in wheat crops, in this study the yield is found to increase when chlorophyll contents increase (Table 4) showing a strong positive relationship between leaves chlorophyll and dry matter accumulation in the spike as observed by Gautam et al. [43].

Carotenoid was found to be decreasing under 5 days of irrigation in this study. It was observed that when chlorophyll is increasing in both growth stages carotenoid is decreasing. This shows water stress increased the level of carotenoids in the varieties, these findings can be tributed to the fact that carotenoid is found to play a significant role in osmotic adjustment during water stress conditions. Just like proline, carotenoid is found to be accumulated in plants in response to water stress [42,44]. The negative correlation found between carotenoid and chlorophyll A and B contents can be linked to the complementary role

among photosynthetic pigments and their role in determining the dry matter contents in crops as observed by Bijanzadeh and Emam [41] and Nezhadahmadi et al*.* [45]. Carotenoids being the secondary pigment might be increasing when the primary pigment (chlorophyll) is reducing in other to counter the ill-effect of reduced chlorophyll caused by water stress. This may probably be the reason why the varieties (ATTILA-7, KAUZ-9, and TEVEE'S) with higher carotenoids under 15 days irrigation gave better yield compared to those with less quantity of carotenoids (Fig. 6). This gives a good reason why carotenoid contents of a variety growing under water stress should be consider as a traits to be targeted by breeders when improving wheat crops in dry areas.

Correlation analysis among the photosynthetic pigments and yield-related traits and yield show that grain yield is significantly correlated with all the traits which were all found to be increasing with an increase in irrigation. A similar observation was reported by Fotovat et al. [46]. However, the correlation between carotenoids and grain yield was negative.

This study shows all the varieties produced more yield under 5 days irrigation regime as compared to 15 days irrigation. However, the varieties KAUZ-9, ATTILA-7, and TEVEE'S that have a higher level of chlorophyll under 5 days and high carotenoids under 15 days irrigation show more resistance to water stress and produced more grains compared to others. On the other hand, varieties ATTILA-50, GOUMRIA, NORMAN, SERI-82, and USHER gave the lowest level of both chlorophyll and carotenoid at both growth stages and were also found to produce the least grain.

5. CONCLUSION

Water stress affects wheat crops, from this study we found out that contents of chlorophyll and carotenoids in wheat crops is affected by water stress and changes with growth stage. This in turn reduced the yield in the crop. The contents of the photosynthetic pigments change significantly with an increase in irrigation interval from 5-15 days. The results also show the ability of wheat crops in accumulating carotenoids in response to stress. Results indicated that water stress is more severe during the reproductive stage in wheat compared to the vegetative stage. In conclusion proper manipulation of chlorophyll and carotenoids will increase the grain yield of these varieties under water stress conditions based on the findigns of this study. From the results, the varieties KAUZ-9, ATTILA-7, and TEVEE'S are the most promising varieties among the ten (10) studied varieties because they proved to have a better yield under both 5 days and 15 days irrigation regimes compared to the orther varieties. On the other hand, the varieties, ATTILA-50, GOUMRIA, NORMAN, and SERI-82, were the least performing varieties based on the results obtained from this study.

6. RECOMMENDATIONS

From the results obtained, breeding for increased chlorophyll contents during water stress will surely improve yield in tropical wheat crops, because it has been found to positively correlate with yield, and is therefore recommended as a water stress indicator in tropical wheat. Reduced amount of chlorophyll under water stress is also an indicator of increased carotenoid. A higher level of carotenoids under stress conditions has stood to be good traits in wheat that helps them to tolerate water stress, is therefore recommended when breeding for drought stresstolerant. The varieties KAUZ-9, ATTILA-7, AND TEVEE'S should be planted in a water deficit environment since they were found to have better yield under both irrigation regimes compared to others.

Since response of the plant to water stress in the soil can't be linked to a single trait, studying the relationship of several traits under water stress is recommended for a better understanding of the whole water stress tolerance in the tropical wheat varieties.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declared that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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